

MOLECULAR PHYLOGENETIC REVISION OF THE FRESHWATER
LIMPET GENUS *FERRISSIA* (PLANORBIDAE: ANCYLINAE)
IN NORTH AMERICA YIELDS TWO SPECIES:
FERRISSIA (*FERRISSIA*) *RIVULARIS* AND *FERRISSIA* (*KINCAIDILLA*) *FRAGILIS*

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ABSTRACT

Members of the gastropod genus *Ferrissia* Walker, 1903, have a near-cosmopolitan distribution in freshwater ecosystems. Five North American species, distinguished by conchological, habitat, and distributional details, are generally recognized. However, they can be difficult to diagnose in practice, and we aimed to comprehensively revisit their systematics and taxonomy using molecular phylogenies. Our primary result was congruent for all three genetic markers: two highly distinctive *Ferrissia* clades, each encompassing multiple nominal species, were widely distributed across North American watersheds in both lentic and in lotic habitats. One clade was restricted to North America and was exclusively composed of lotic *F. rivularis* (Say, 1817) and lentic *F. parallela* (Haldemann, 1841). These two taxa exhibited geographic, rather than taxonomic, genetic structuring, strongly implying that they are conspecific and that *F. parallela* is a junior synonym of *F. (Ferrissia) rivularis*. The other clade contained three North American taxa, *F. fragilis* (Tryon, 1863) and putative specimens of *F. mcneilli* Walker, 1925, and *F. walkeri* (Pilsbry & Ferriss, 1907), in addition to the Hawaiian *F. sharpi* (Sykes, 1900), as well as multiple invasive Eurasian founder populations. All four taxa shared overlapping genotypes and shell phenotypes, prompting us to conclude that they are conspecific with *F. fragilis* having taxonomic priority. This New World species had robust phylogenetic links to Old World congeners, thereby enabling us to unite global septum-forming *Ferrissia* species under the subgenus *Kincaidilla* Hannibal, 1912. *Ferrissia rivularis* and *F. fragilis* differed in a number of conchological traits, including maximum body size, details of apex positioning and orientation, and the ability to form a septum. The two former traits were sufficient to distinguish sympatric populations.

Key words: biodiversity, gastropod, freshwater, limpet, phylogeny, taxonomy.

INTRODUCTION

North American freshwater limpets have been relatively understudied since Paul Basch's classic body of work (Basch, 1959, 1960, 1962a, b, 1963a, b). Taxonomic uncertainty among ancylinid limpets, by far the most widespread and common constituent group, has contributed to this neglect. It pervades the literature and it is quite common for ancylinid study taxa to be identified to generic (Morgan et al., 2002) or familial/subfamilial (Stewart et al., 1998; McMahon, 2004) status only. This unsatisfactory situation stems in part from inadequate taxonomic descriptions (Basch, 1963a; Jokinen, 1978; McMahon, 2004) – see Burch (2009)

for a detailed compilation of original species descriptions – exacerbated by pronounced conchological plasticity (Basch, 1963a, b; Russell-Hunter et al., 1981; McMahon, 2004; Hovingh, 2004; Dillon & Herman, 2009).

A number of recent molecular phylogenetic studies have helped put ancylinid systematics and taxonomy on a firmer footing, for example, excluding convergent freshwater limpet lineages (Albrecht et al., 2004), confirming the linkage with planorbids (Morgan et al., 2002; Albrecht et al., 2004; Walther et al., 2006a), and detecting basal New World and Holarctic sister clades (Walther, 2006a). The phylogenetic placement of ancylinids within Planorbidae, in association with earlier anatomical work (Hubendick, 1978),

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TABLE 1. Morphology and habitat of the five nominal species of North American *Ferrissia* recognized by Basch (1963).

Nominal species	Shell characteristics	Habitat & Distribution	Type Locality
<i>F. rivularis</i> (Say, 1817)	Robust, ≤ 7 mm, elevated, elliptical aperture, apex in midline or slightly to the right.	Rivers and streams across the U.S.	Unknown rivulets
<i>F. parallela</i> (Haldeman, 1841)	Thin-shelled, large, ≤ 9 mm, elevated and very narrow, peritreme often arched, obtuse apex in midline.	Lakes, northern states and Canada	New England
<i>F. fragilis</i> (Tryon, 1863)	Fragile & minute, ≤ 4 mm, moderately elevated or depressed, oval aperture, can form a septum, sub-acute or obtuse apex in right-posterior quadrant.	Small bodies of standing water, often temporary, across the U.S.	Laguna Honda, California
<i>F. walkeri</i> (Pilsbry & Ferriss, 1907)	Thin-shelled, ≤ 6 mm, usually depressed, oval aperture, apex subacute, often far into the posterior-right quadrant.	Ponds and lakes, Arkansas, Michigan, southern California	Leafy fish pond in Rogers, Benton Co., Arkansas
<i>F. mcneilli</i> Walker, 1925	Fragile, ≤ 5 mm, very much depressed, often a glossy red-brown color, apex a rounded bump in right-posterior quadrant.	Streams, Mobile Bay drainage, Alabama	Mandeville Creek, Mobile Co., Alabama

has prompted the taxonomic revision of these limpets from familial (Ancylidae) to subfamilial (Ancylinae) status (Albrecht et al., 2007; Strong et al., 2008). Molecular phylogenies have also proven useful for ancylinid biodiversity studies, for example, taxonomic revision of the genus *Laevapex* (Walther et al., 2006a) and detection of cryptic endemic (Pfenninger et al., 2003; Albrecht, 2006) and invasive (Walther et al., 2006b, c) species.

The ancylinid genus *Ferrissia* Walker, 1903, has been recorded from lentic and lotic freshwater habitats across North America and on every continent except Antarctica (Basch, 1963a; Hubendick, 1964; Clarke, 1973; Gómez et al., 2004; Thiengo et al., 2004). The genus is characterized by an apical microsculpture of fine radiating grooves formed during embryogenesis (Walker, 1903; Basch, 1963a; Hubendick, 1964), a flagellum-bearing simple penis (Hoff, 1940; Basch, 1963a; Hubendick, 1964), and a single-lobed pseudobranch that bears the anus (Hoff, 1940; Basch, 1963a). Hubendick (1964) established New World and Old World subgenera (respectively *Ferrissia* and *Pettancylus*) based on details of penial complex morphologies.

Basch (1963a) synonymized over 20 nominal North American *Ferrissia* taxa into five species

(*F. rivularis*, *F. parallela*, *F. fragilis*, *F. walkeri*, and *F. mcneilli*) distinguished by details of their shell morphologies and their habitats and distributions (Table 1). He was very aware of ecophenotypic conchological variation and the potential difficulty this posed for diagnoses based on fine-scale conchological features (Basch, 1963b). Consequently, habitat fidelities (lotic for *F. rivularis* and *F. mcneilli*; lentic for *F. fragilis*, *F. parallela* and *F. walkeri*) are critical attributes of his taxonomic scheme. However, Basch (1963a) was open to the possibility of evolutionary transition among habitats, speculating that the lentic taxa *F. parallela* and *F. walkeri* may have evolved from an ancestral lotic *F. rivularis* lineage. With a few minor exceptions, for example, *F. fragilis* synonyms (Thompson, 1999), Basch’s (1963a) taxonomy of North American *Ferrissia* has generally remained widely accepted (Clarke, 1973; Jokinen, 1978; Hovingh, 2004; Fritz & Feminella, 2006; Pyron et al., 2008). Nevertheless, multiple authors report inconsistencies, including the occurrence of species morphotypes in ostensibly ectopic habitats – e.g., lotic *F. walkeri* (Hovingh, 2004) and *F. fragilis* (Pyron et al., 2008 – and the difficulty of distinguishing *F. fragilis* and *F. walkeri* (Jokinen, 1978), a shortcoming also acknowledged by Basch (1963a).

A small number of *Ferrissia fragilis*, *F. rivularis* and *F. parallela* have been included in previous molecular phylogenetic studies (Walther et al., 2006a, b, c). All three taxa placed in a robust ancylinid clade comprising the genera *Ferrissia*, *Rhodacmea*, and *Ancylus*. However, the three congeners were not monophyletic: *F. rivularis* and *F. parallela* exhibited a very close phylogenetic relationship, but *F. fragilis* was topologically disjunct, being either weakly sister to *Rhodacmea* and *Ancylus* or forming part of a basal ancylinid polytomy (Walther et al., 2006a, b). *Ferrissia fragilis* appears to lack North American sister taxa and has complex phylogenetic ties to Old World *Ferrissia* populations, including an apparent sister relationship with African *F. cf. clessiniana* (Jickeli, 1882) (Walther et al., 2006b), as well as cryptic, and frequently misidentified, invasive founder populations in Europe and Asia (Walther et al., 2006b). In contrast with the above studies,

Dillon & Herman (2009) recently proposed synonymization of *F. rivularis* and *F. fragilis* based on allozymic and morphometric analyses of two South Carolina lotic populations, one from a fast flowing river, the other a languid creek. They reached this conclusion despite acknowledging that exemplar specimens forwarded to us for genotyping from both of their study populations placed unambiguously within the *F. fragilis* clade (Dillon & Herman, 2009).

Our goal in this study was to revisit North American *Ferrissia* taxonomy using molecular phylogenetic datasets generated from an extensive sampling of continental populations. Our data indicate that Basch's (1963a) taxonomic scheme requires further consolidation: only two species, *F. rivularis* and *F. fragilis*, appear to be phylogenetically supportable. Both taxa are widespread in continental lentic and lotic habitats, and we provide guidelines for distinguishing them morphologically.

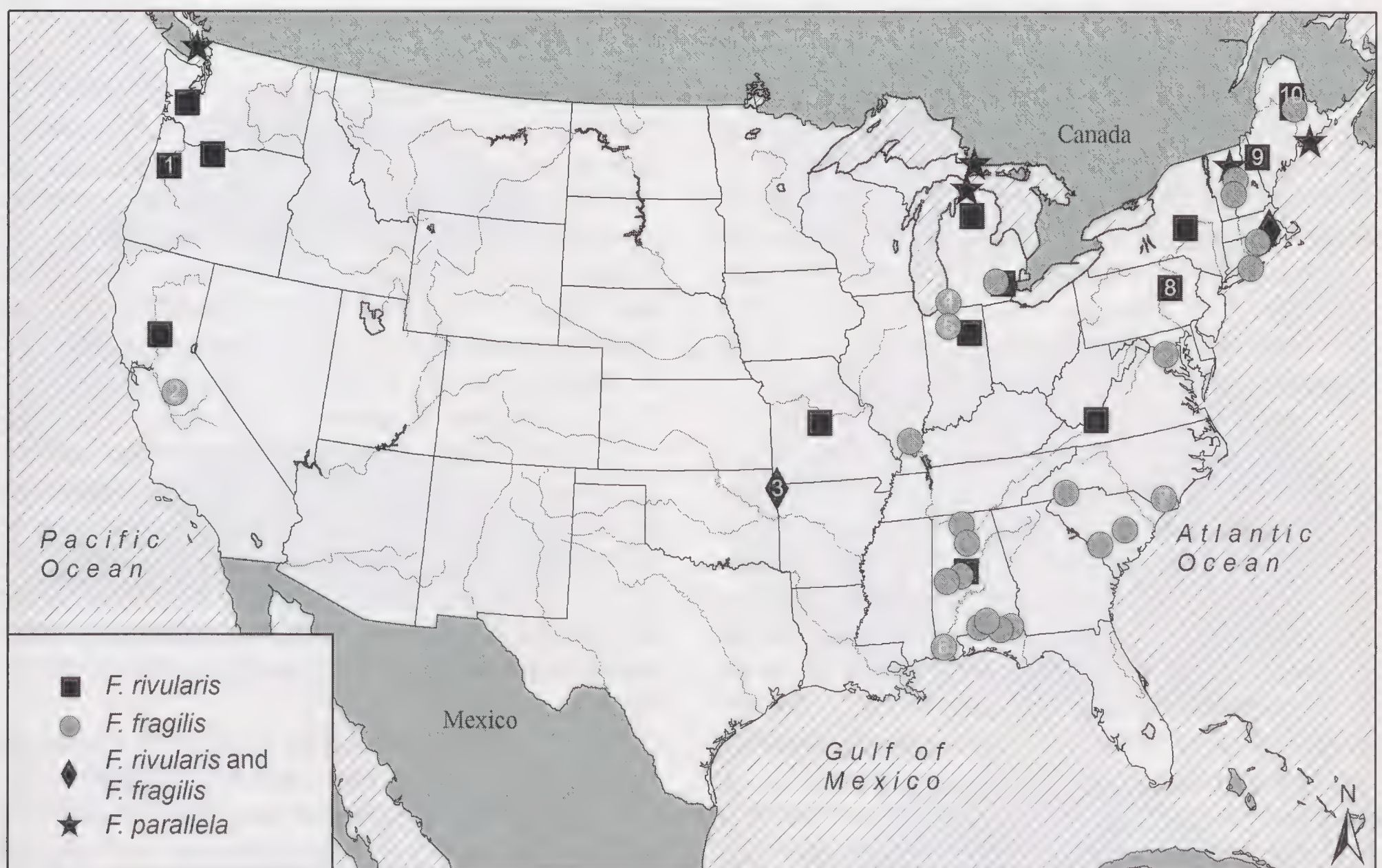


FIG. 1. Map showing the approximate locations of our North American *Ferrissia* species sampling localities; Table 2 has detailed locality information. Samples were identified using Basch's (1963a) morphological criteria, but note that we were unable to unambiguously identify specimens of *Ferrissia walkeri* or *F. mcneilli*. Sampling sites at, or near, type localities are identified numerically: 1 = *Ancylus oregonensis* (syn. *F. rivularis*), 2 = *Gundlachia californica* (syn. *F. fragilis*), 3 = *A. walkeri* (syn. *F. walkeri*), 4 = *F. michiganensis* (syn. *F. walkeri*), 5 = *F. bartschi* (syn. *F. fragilis*), 6 = *F. mcneilli*, 7 = *Ancylus* (*F.*) *hendersoni* (syn. *F. fragilis*), 8 = presumed *F. rivularis* type locality, 9 = *Ancylus ovalis* (syn. *F. rivularis*), 10 = *Ancylus borealis* (syn. *F. rivularis*).

MATERIALS AND METHODS

Specimen Collection

Ferrissia populations were sampled from a diversity of lentic and lotic habitats throughout the eastern U.S. as well as from Pacific Coast drainages from California to British Columbia (Fig. 1; Table 2). Our initial aim to collect from topotypic populations was compromised by either an absence of meaningful type locality information (*F. rivularis* and *F. parallela*; Table 1), or vague locality information coupled with extensive subsequent landscape changes that rendered it impossible to confidently resample the actual type localities (*F. fragilis*, *F. mcneilli* and *F. walkeri*; Table 1). We ameliorated these shortcomings by obtaining samples from: (1) populations as close to type localities as possible (e.g., Benton County, Arkansas, ponds for *F. walkeri*); (2) still tractable sites where the target taxon had been reliably sampled, e.g., Eslava Creek, Alabama, the source of UMMZ 161631 *F. mcneilli* sampled by McNeill and identified by Walker; (3) type localities of *F. fragilis*, *F. rivularis* and *F. walkeri* synonyms (Fig. 1; Table 2). We also incorporated new extra-continental samples of *Ferrissia* (the putative endemic *F. sharpi* from Hawaii and suspected invasive *F. fragilis* from the Azores and France), as well as a North American ancylinid, *Hebetancylus excentricus* that had been unavailable during our previous studies (Walther et al., 2006a, b, c). All specimens were preserved in 95% ethanol.

Individual limpets were assigned to the genus *Ferrissia* based on their possession of diagnostic radial apical microsculpture (Basch, 1963a; Burch, 1988), and species identifications were made based on available keys and descriptions (Basch, 1963a; Table 1), in addition to comparison with holotype material (Fig. 2). Note, however, that the *F. parallela* holotype is much smaller than the maximum adult size (9 mm in length; Basch, 1963a) for this species, and the *F. fragilis* holotype is partially damaged (Fig. 2). Basch (1963a) emphasized that his *Ferrissia* key applied to adult specimens only and that sub-adults might well prove intractable. We concur: his key readily identified larger specimens of *F. rivularis* in rivers, *F. parallela* from northern ponds and lakes and *F. fragilis* from ponds and lakes throughout the U.S. Small specimens proved more difficult, and many of the limpets < 4 mm in length sampled from the slower-moving sections of creeks and rivers resembled *F. fragilis*. We had significant problems identify-

ing unambiguous members of the remaining two species. Basch's (1963a) description of *F. mcneilli* is perfunctory and includes no diagnostic characters to distinguish it from *F. fragilis* except habitat: respectively Mobile, Alabama, area streams versus ponds/ditches continent-wide. Our Mobile, Alabama, stream samples matched Basch's (1963a) description of *F. fragilis*. Basch (1963a) differentiated *F. walkeri* from *F. fragilis* by the former's larger size and habitat of larger bodies of standing water – however, the former's type locality is a pond (Table 1) – but we concur with Jokinen (1978) that the species descriptions overlap and that it is very difficult to confidently distinguish these two taxa. Our samples from *F. walkeri* near-topotypic (Benton County, Arkansas) and toposyntypic (Berrien County, Michigan) locations best fit Basch's (1963a) *F. fragilis* description, and none of our extensive sampling of North American *Ferrissia* populations have, to date, recovered unambiguous specimens of *F. walkeri*.

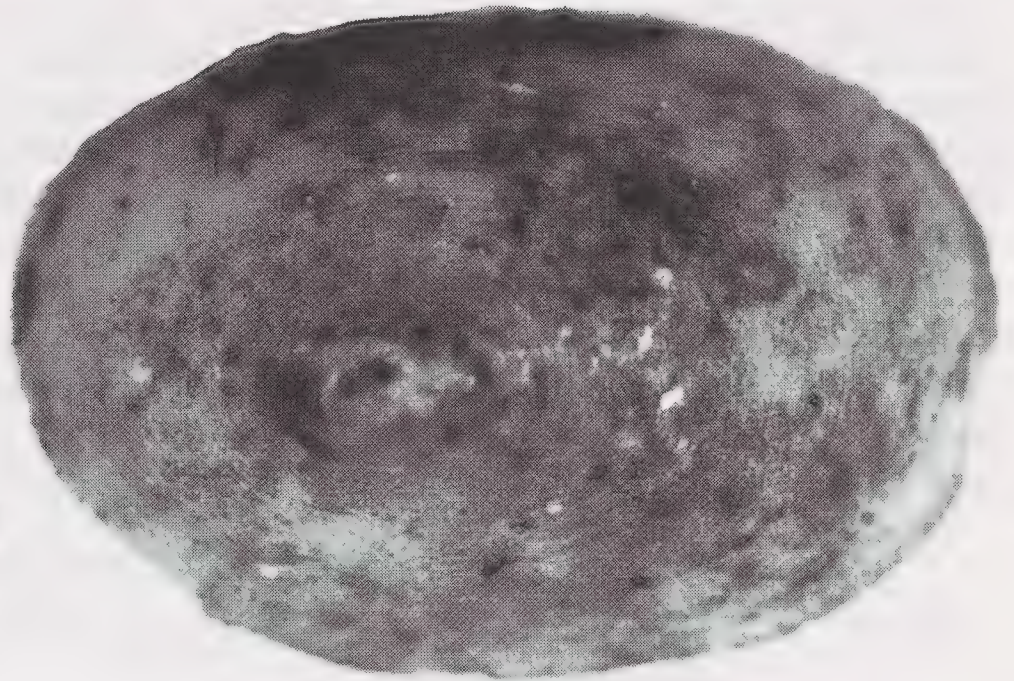
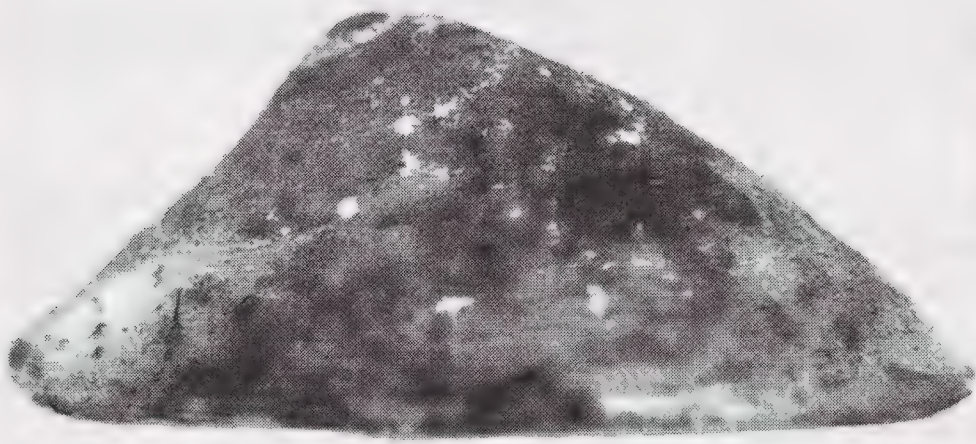
Molecular & Phylogenetic Methods

DNA was extracted and target fragments of three genes – 808 nt (aligned) of nuclear ribosomal large-subunit (28S), 639 nt (aligned) of nuclear ribosomal second internal transcribed spacer (ITS-2) and 671 nt of mitochondrial mt cytochrome oxidase I (COI) – were amplified and directly sequenced (both strands) using the molecular techniques and primer pairs detailed in Walther et al. (2006a). Edited sequences for each gene fragment were aligned using ClustalW (Thompson et al., 1994), implemented in Sequence Navigator (Applied Biosystems, Foster City, California), and refined manually. Novel genotypes were assembled in a NEXUS format using Sequence Monkey 2.9.0 (available at http://sequence_monkey.tripod.com) and they have been deposited in GenBank (GU391035–GU391217).

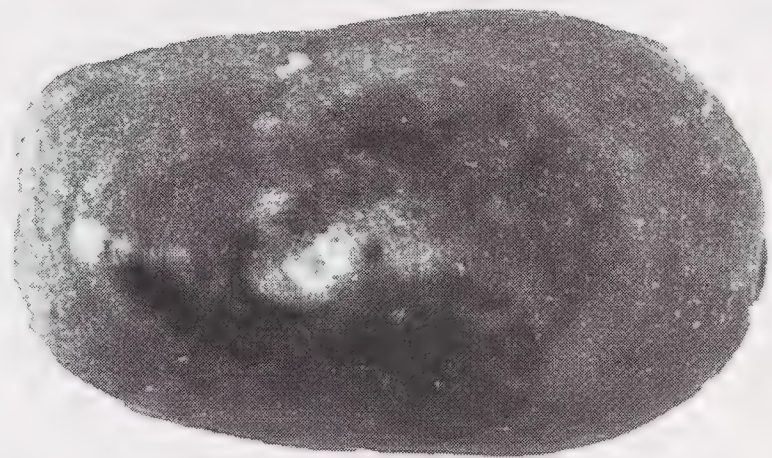
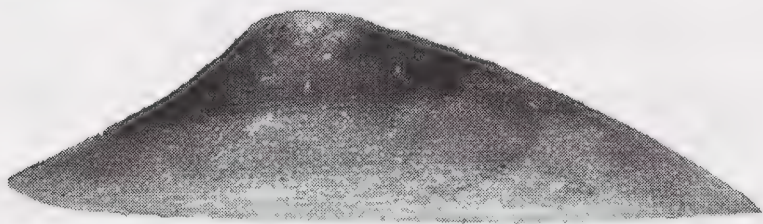
PAUP* 4.0b10 (Swofford, 2003) and MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003) were employed for phylogenetic analyses. Maximum parsimony searches were heuristic, with equal character weighting, inferred sequence gaps coded as missing data, 100 random stepwise additions, and tree-bisection-reconnection (TBR) branch-swapping. Bootstrap (Felsenstein, 1985) branch support (BS) levels were estimated with 100 replicates, 100 random additions each. The nearest-neighbor interchange (NNI) branch-swapping algorithm was employed for generating ITS-2 bootstrap support, though

F. rivularis
ANSP 21982

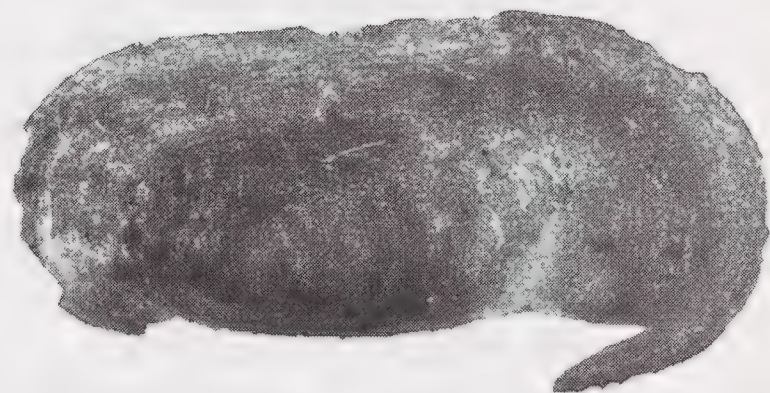
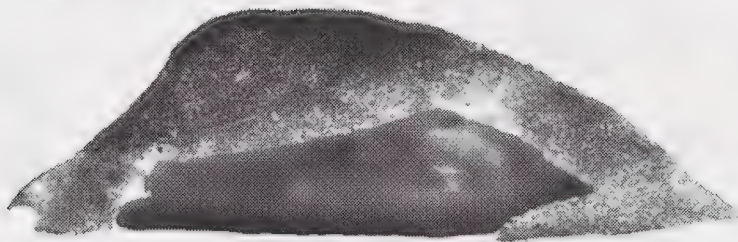
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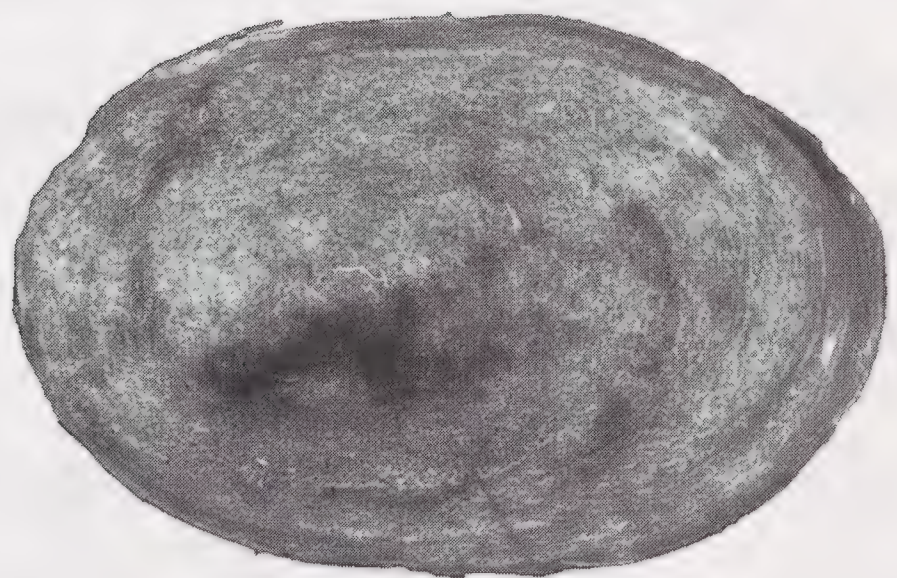
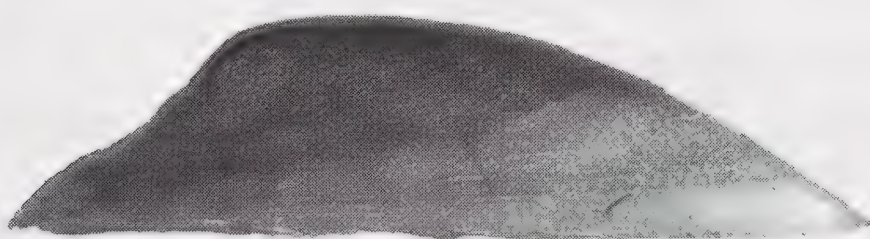
F. parallela
ANSP 21996



F. fragilis
ANSP 22011



F. walkeri
ANSP 87479



F. mcneilli
UMMZ 161631



FIG. 2. Lateral and dorsal photographs of museum type and reference shells for the five North American *Ferrissia* species recognized by Basch (1963a). The *F. parallela*, *F. fragilis*, and *F. walkeri* specimens shown are holotypes; the *F. rivularis* specimen is a syntype; and the *F. mcneilli* was collected by L. H. McNeill near the taxon's type locality and identified by Walker.

TABLE 2. Taxonomy, sampling location, location code, genotype information (# individuals sequenced/# unique genotypes obtained), and voucher information (University of Michigan Museum of Zoology [UMMZ] number, unless otherwise noted) for the ancylinid study populations. North American taxa were identified using Basch’s (1963a) morphological criteria, but note that we were unable to unambiguously identify specimens of *Ferrissia walkeri* or *F. mcneilli*. Samplings sites at, or near, type localities are indicated in bold.

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
<i>Ferrissia rivularis</i>	Black River, northeast of Littlerock, Thurston Co., Washington [46.9186°N; 123.0011°W]	WAB	-	2/1	1/1	301123
	Black River, just south of Mumby, Thurston Co., Washington [46.8678°N; 123.0178°W]	WAC	1/1	3/2	1/1	301124
	Black Lake Ditch, west of Tumwater, Thurston Co., Washington [47.0017°N; 122.9525°W]	WAD	-	2/1	1/1	301125
	Black River, Littlerock, Thurston Co., Washington [46.9014°N; 123.0172°W]	WAL	-	4/1	1/1	301126
	Small tributary (of Black River?), north of Little-rock, Thurston Co., Washington [46.9186°N; 123.0178°W]	WAS	-	4/3	-	301127
	Battle Creek south of Salem, Marion Co., Oregon [44.8514°N; 123.0517°W] *near <i>Ancylus oregonensis</i> (syn. <i>F. rivularis</i>) type locality	ORB	-	4/2	-	301128
	Mill Creek in The Dalles, Wasco Co., Oregon [45.6006°N; 121.1853°W]	ORL	3/1	12/3	6/2	301204, 301205
	Mill Creek, southwest of The Dalles, Wasco Co., Oregon [45.5678°N; 121.2342°W]	ORM	1/1	5/3	1/1	301129
	Pudding River, east of Salem, Marion Co., Oregon [44.9511°N; 122.8667°W]	ORP	-	4/2	-	301130
	Willamette River, southwest of Salem, Marion Co., Oregon [44.8675°N; 123.1353°W]	ORW	1/1	5/2	1/1	301131
	Feather River at Oroville Wildlife Area, south of Oroville, Butte Co., California [39.4511°N; 121.6022°W]	CAF	1/1	1/1	2/1	301132
	Missouri, USA	MOA	-	-	5/1	301179
	Little River, Huntington, Huntington Co., Indiana [40.8681°N; 85.5011°W]	INL	1/1	1/1	1/1	301178
	AuSable River, Crawford Co., Michigan [44.6648°N; 84.6419°W]	MIA	1/1	1/1	1/1	300224
	Fleming Creek, Washtenaw Co., Michigan [42.3050°N; 83.6617°W]	MIF	-	5/2	-	300280
	Small tributary entering Crystal Lake, Benton Co., Arkansas [36.3352°N; 94.4338°W]	ARL	3/1	-	3/1	301194
	Little Cahaba River, Bibb Co., Alabama [33.0545°N; 86.9703°W]	ALK	1/1	2/1	-	300226
	Oaks Creek, Toddsville, Otsego Co., NY [42.8670°N; 74.9572°W]	NYO	-	2/2	2/1	301134

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Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Briar Creek, just west of Berwick, Columbia Co., Pennsylvania [41.0456°N; 76.2851°W]	PAB	1/1	1/1	1/1	301133
	Susquehanna River, Watson town, Northumberland Co., Pennsylvania [41.0773°N; 76.8587°W] *presumed <i>Ancylus rivularis</i> (syn. <i>F. rivularis</i>) type locality, according to Basch(1963a)	PAS	1/1	1/1	1/1	301135
	New River, Giles Co., Virginia [37.3150°N; 80.6433°W]	VAN	-	1/1	-	300225
	Androscoggin River, West Bethel, Oxford Co., Maine [44.4068°N; 70.8612°W] *near <i>Ancylus ovalis</i> (syn. <i>F. rivularis</i>) type locality	MEA	-	4/2	-	301136
	Fish Stream, Patten, Penobscot Co., Maine [45.9925°N; 68.4413°W]	MEF	-	1/1	1/1	301137
	Rockabema Stream, just southwest of Medway, Penobscot Co., Maine [45.5946°N; 68.5482°W]	MEO	1/1	5/2	1/1	301138
	Rowe Brook, just west of Patten, Penobscot Co., Maine [46.0013°N; 68.4784°W] *near <i>Ancylus borealis</i> (syn. <i>F. rivularis</i>) type locality	MER	1/1	3/2	1/1	301139
	Sweets Pond, Mansfield, Bristol Co., Massachusetts [41.9895°N; 71.2550°W]	MAS	1/1	-	-	301201
<i>F. parallela</i>	Pond on North Pender Island, British Columbia, Canada [48.7358°N; 123.2356°W]	BC	3/1	1/1	1/1	301181
	Douglas Lake, Cheboygan Co., Michigan [45.5797°N; 84.6713°W]	MIL	1/1	1/1	1/1	300229
	Duck Island, Chippewa Co., Michigan [46.3667°N; 84.1347°W]	MID	-	4/1	-	301180
	Joe's Pond, West Danville, Caledonia Co., Vermont [44.4096°N; 72.2019°W]	VTJ	1/1	2/1	1/1	301140
	Kettle Pond, Lanesboro, Caledonia Co., Vermont [44.2964°N; 72.3077°W]	VTK	1/1	4/2	1/1	301141
	Beaver Dam Pond in Acadia National Park, Mt. Desert Island, Hancock Co., Maine [44.3623°N; 68.1956°W]	MEB	1/1	4/1	-	301142
	Hamilton Pond, Mt. Desert Island, Hancock Co., Maine [44.4273°N; 68.2843°W]	MEH	1/1	4/1	1/1	301143
<i>F. fragilis</i>	Edendale Creek, north of Merced, Merced Co., California [37.4189°N; 120.5011°W]	CAE	-	4/2	-	301144

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Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Merced River at Henderson Park, north of Merced, Merced Co., California [37.5175°N; 120.4172°W] *near <i>Gundlachia californica</i> (syn. <i>F. fragilis</i>) type locality	CAM	1/1	4/2	1/1	301145
	Yosemite Lake, northeast of Merced, Merced Co., California [37.3683°N; 120.4178°W]	CAY	-	5/3	-	301146
	Mexico, Veracruz, Xalapa, Jardin Botanico Clavijero	MXV	1/1	-	1/1	UGSB 1206
	Small creek above Burden Falls at Shawnee National Forest, Illinois [37.5634°N; 88.6422°W]	ILB	-	-	4/1	301182
	Pickerel Lake, Washtenaw Co., Michigan [42.4097°N; 83.9838°W]	MIP	1/1	10/2	2/1	300227, 301174
	Stream west of Harbert, Berrien Co., Michigan [41.8733°N; 86.6371°W] *at <i>F. michiganensis</i> (syn. <i>F. walkeri</i>) type locality	MIW	-	3/1	5/1	301184
	Small tributary entering Crystal Lake, Benton Co., Arkansas [36.3352°N; 94.4338°W] *near <i>Ancylus walkeri</i> (syn. <i>F. walkeri</i>) type locality	ARL	2/1	1/1	1/1	301193
	Lake Maxinkuckee, Culver, Indiana [41.1839°N; 86.3850°W] *<i>F. bartschi</i> (syn. <i>F. fragilis</i>) type locality	INM	1/1	-	-	301176
	Big Creek, south of Dothan, Houston Co., Alabama [31.0730°N; 85.4127°W]	ALA	-	2/2	2/1	301154
	Cahaba River at Booth's Ford, Shelby Co., Alabama [33.1853°N; 87.0015°W]	ALB	1/1	2/1	-	300192
	Cahaba River at CR 52 Crossing, Shelby Co., Alabama [33.2842°N; 86.8828°W]	ALC	-	2/2	-	300193
	Cahaba River, Centreville, Bibb Co., Alabama [32.9448°N; 87.1405°W]	ALD	-	1/1	1/1	301155
	Eslava Creek, Mobile Co., Alabama [30.6793°N; 88.1464°W] *near <i>F. mcneilli</i> type locality	ALE	-	1/1	1/1	301164
	Mouth of Eslava Creek at Eslava Lake, Mobile Co., Alabama [30.6787°N; 88.1529°W] *near <i>F. mcneilli</i> type locality	ALF	1/1	1/1	1/1	301166
	Big Brush Creek, Wedgeworth, Hale Co., Alabama [32.8172°N; 87.7506°W]	ALG	-	2/1	2/2	301156
	Conecuh River, between Fairfield and Brooklyn, Covington Co., Alabama [31.1856°N; 86.6689°W]	ALH	-	2/1	2/2	301157

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Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Limestone Creek, Limestone Co., Alabama [34.6269°N; 86.8840°W]	ALL	-	1/1	1/1	301158
	Choctawhatchee River, Eunola, Geneva Co., Alabama [31.0294°N; 85.8538°W]	ALR	-	2/1	2/1	301159
	Yellow River, Covington Co., Alabama [31.3577°N; 86.3421°W]	ALY	-	3/3	2/1	301160
	Mulberry Fork of Black Warrior River, Bangor, Blount Co./Cullman Co., Alabama [33.9975°N; 86.7494°W]	ALZ	-	-	1/1	301161
	Lake Waccamaw, NC, USA [34.3066°N; 78.4957°W] *<i>Ancylus (F.) hendersoni</i> (syn. <i>F. fragilis</i>) type locality	NCW	-	-	2/2	301175
	North Saluda River at Les Mullinax County Park, northeast of Lima, Greenville Co., South Carolina [35.1271°N; 82.4264°W]	SCA	2/1	-	2/1	301187
	Potato Creek, southwest of Davis Station, Clarendon Co., South Carolina [33.5735°N; 80.2926°W]	SCP	2/1	-	2/1	301183
	Salkehatchie River, Barnwell Co., South Carolina [33.2370°N; 81.4020°W]	SCS	-	1/1	-	300228
	Laurel Lake, just west of Mattituck, Suffolk Co., New York [40.9778°N; 72.5562°W]	NYL	1/1	2/1	-	301147
	Lake Morey, Fairlee, Orange Co., Vermont [43.9264°N; 72.1417°W]	VTL	1/1	1/1	1/1	301148
	Mill Pond, Windsor, Windsor Co., Vermont [43.4761°N; 72.3976°W]	VTM	1/1	1/1	1/1	301149
	Sweets Pond, Mansfield, Bristol Co., Massachusetts [41.9895°N; 71.2550°W]	MAS	1/1	1/1	-	301200
	Quinebaug River, Plainfield, Windham Co., Connecticut [41.7471°N; 71.9142°W]	CTQ	-	1/1	1/1	301150
	Penobscot River, northwest of Medway, Penobscot Co., Maine [45.6149°N; 68.5392°W]	MEP	1/1	2/1	-	301151
	Little Falls Branch, Maryland [38.9441°N; 77.1169°W]	MDL	-	1/1	6/1	301186
	Impoundment of North Mosquito Creek at Cypress Cove Nature Park, Gadsden Co., Florida [30.7358°N; 84.8047°W]	FLM	-	1/1	1/1	301185
	São Miguel Island, Azores	AZOR	1/1	1/1	1/1	301196
	Basin of Arcachon, south of Le Teich, France	France	-	4/1	-	301197
	Subsidence pond in Upper Silesia, southern Poland	Poland	-	4/1	-	300279
	Chi-Chi, Nantou Co., Taiwan	Taiwan	-	2/1	2/1	300278
	Alimodian, Iloilo Prov., Panay Island, Philippines	Philippines	-	2/1	2/1	300276

(continues)

(continued)

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
<i>F. sharpi</i>	Lulumahu Stream, Nuuanu Valley, Oahu, Honolulu Co., HI [21.3461°N; 157.8209°W]	HIO	-	1/1	1/1	301191
<i>Laevapex fuscus</i>	Osage Creek, Benton Co., Arkansas [36.1972°N; 94.3377°W]	ARO	1/1	-	-	301195
	Marsh at the south end of Lake Maxinkuckee, Culver, Indiana [41.1839°N; 86.3850°W]	INM	2/1	-	-	301177
	Perry Lake at Perry Lakes Park, Marion, Perry Co., Alabama [32.6974°N; 87.2434°W]	ALP	1/1	-	-	301163
	Corn Creek Shoals, Coosa River, Elmore Co., Alabama [32.5519°N; 86.2011°W]	ALS	1/1	-	-	301198
	Pickrel Lake, Washtenaw Co., Michigan [42.4097°N; 83.9838°W]	MIP	1/1	6/1	1/1	300206
	Passaic River near Two Bridges, New Jersey [40.8858°N; 74.2689°W]	NJP	1/1	-	-	301152
	Wading River, Mansfield, Massachusetts [41.9894°N; 71.2548°W]	MAW	1/1	-	-	301153
	Big Cypress Bend, Florida State Park, Collier Co., Florida [25.9418°N; 81.4695°W]	FLB	1/1	-	-	300218
	Lake Helen (Helena), Volusia Co., Florida [28.9850°N; 81.2303°W]	FLH	3/2	-	-	301189
	Impoundment of North Mosquito Creek at Cypress Cove Nature Park, Gadsden Co., Florida [30.7358°N; 84.8047°W]	FLM	6/1	-	-	301185
<i>Hebetancylus excentricus</i>	Eslava Creek, Mobile Co., Alabama [30.6793°N; 88.1464°W]	ALE	-	1/1	-	301165
	Eslava Lake, Mobile Co., Alabama [30.6787°N; 88.1529°W]	ALF	-	1/1	-	301167
	Lake Helen (Helena), Volusia Co., Florida [28.9850°N; 81.2303°W]	FLH	2/1	1/1	-	301188
	Lake Talquin, west of Tallahassee, Leon Co., Florida [30.4389°N; 84.5304°W]	FLT	6/1	-	-	301190
<i>Uncancylus</i> sp. (?)	Lago Ranco, Chile [40.1910°S; 72.2606°W]	CLL	1/1	-	-	UGSB 1201

TBR was used for the other datasets. For maximum likelihood (ML) analyses, the best-fit model for each of the four datasets (TVM+I+G for 28S and COI, TVM+I for ITS-2) was obtained in Modeltest 3.06 (Posada & Crandall, 1998). In each case, one of the MP trees was used as a starting tree with the TBR branch-swapping algorithm in PAUP*. Bootstrap support values were generated using a fast-heuristic search with 100 replicates. Bayesian searches were run for

1x10⁶ generations in MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003) using the same best-fit model as in the ML analyses. Posterior probabilities were calculated by creating a majority-rule consensus for every 100th tree after burn-in of the first 3000 trees using PAUP*. Parsimony networks for three mt COI tip-clades of North American *Ferrissia* were constructed using the statistical parsimony (Templeton et al., 1992) method in TCS 1.21 (Clement et al., 2000).

RESULTS

Molecular Phylogenies

The addition of a large number of novel North American *Ferrissia* 28S nuclear rDNA genotypes to the Walther et al. (2006a) dataset did not result in any significant topological

changes (Fig. 3). The same two *Ferrissia* 28S lineages were recovered: one containing a single genotype common to *F. rivularis* and *F. parallela* specimens sampled across the continent; the other comprised of three genotypes, all recovered from *F. fragilis* specimens, the most common of which was also present in near-topotypic populations of putative *F. mc-*

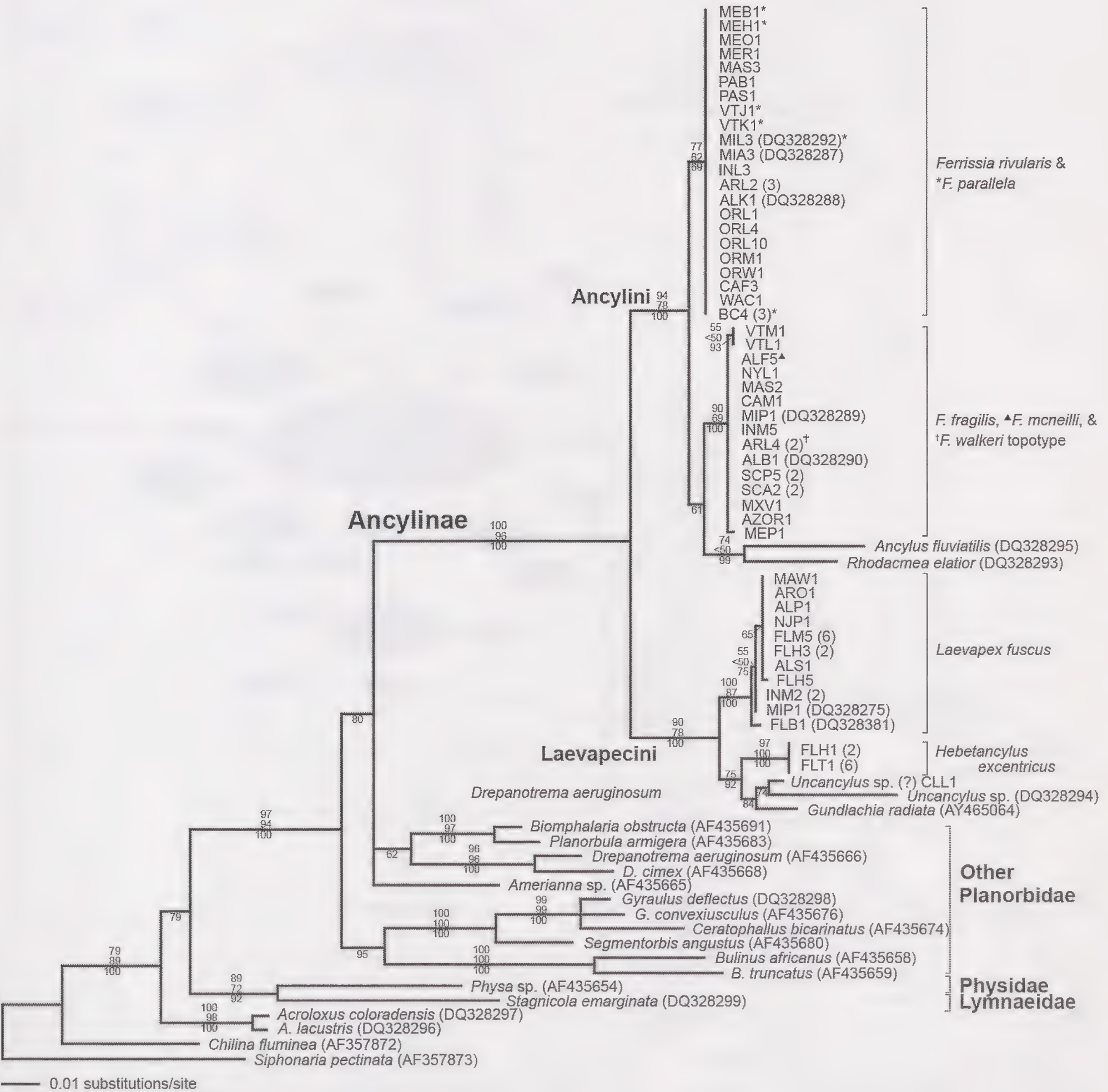


FIG. 3. Bayesian consensus phylogram for our nuclear 28S rDNA dataset. *Siphonaria pectinata* served as the outgroup, and non-novel genotypes obtained from GenBank are indicated by their terminal accession numbers. The taxonomic names and location codes are given for novel ancylinid sequences; Table 2 has detailed sampling location information. If >1 individual/location bore a genotype, this number is indicated in parentheses. In the *F. fragilis* clade, SCA2 (2) represents the two genotyped limpets sourced from Dillon & Herman's (2009) North Saluda River study population. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented.

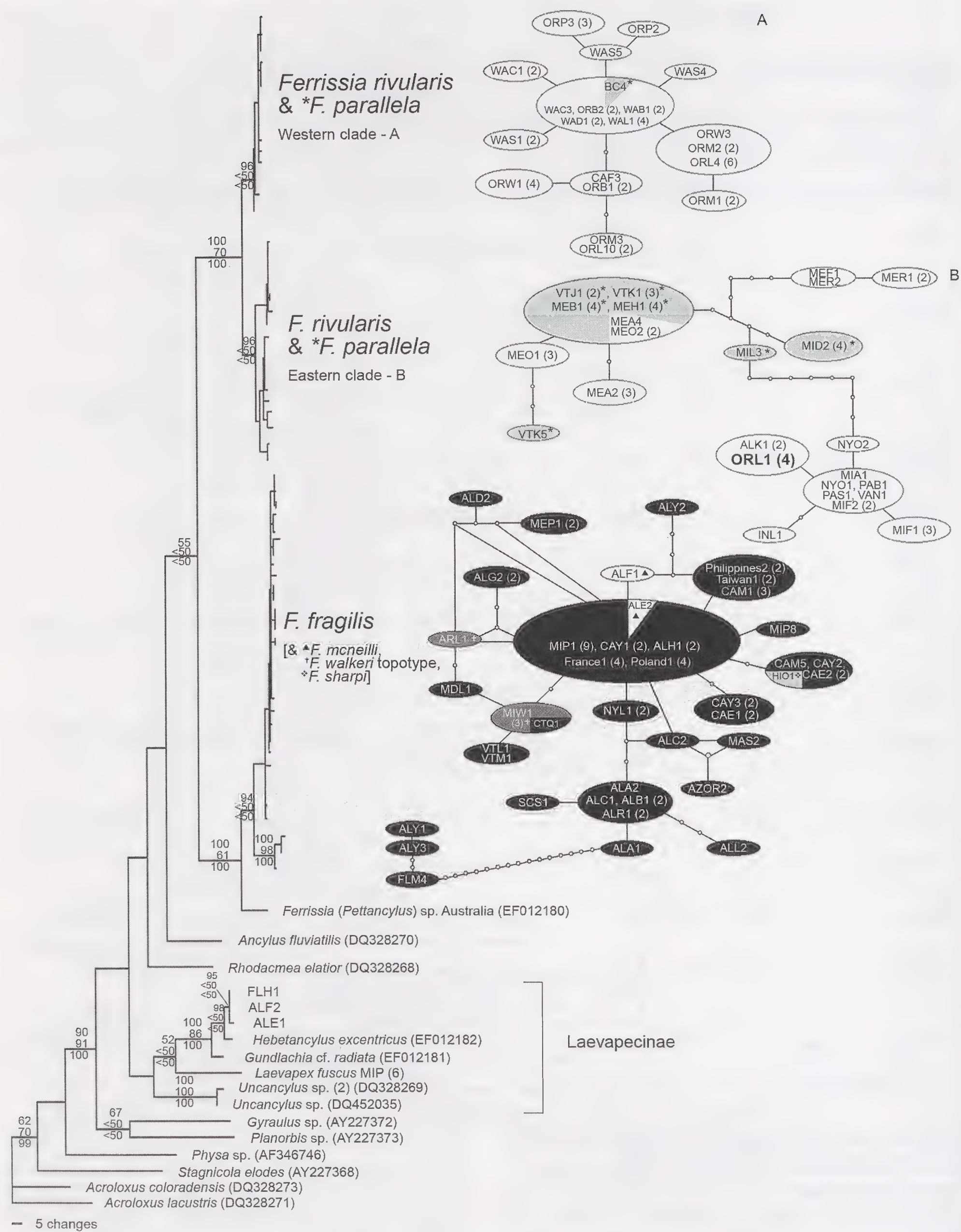


FIG. 4. A maximum parsimony phylogenetic tree for the North American *Ferrissia* species mt COI dataset presented together with unrooted statistical parsimony networks for each of the mt COI tip clades. The tree topology shown was one of the 5000 most parsimonious trees saved, but it only differed in within-tip clade branching order from the strict consensus tree. *Acroloxus lacustris* and *A. coloradensis* served as outgroups. Table 2 has location codes for all novel genotypes. If >1 individual/location bore a genotype, this number is indicated in parentheses. Non-novel genotypes obtained from GenBank are indicated by their terminal accession numbers. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented. In the unrooted networks, the size of each oval corresponds to the total number of individuals sharing a haplotype.

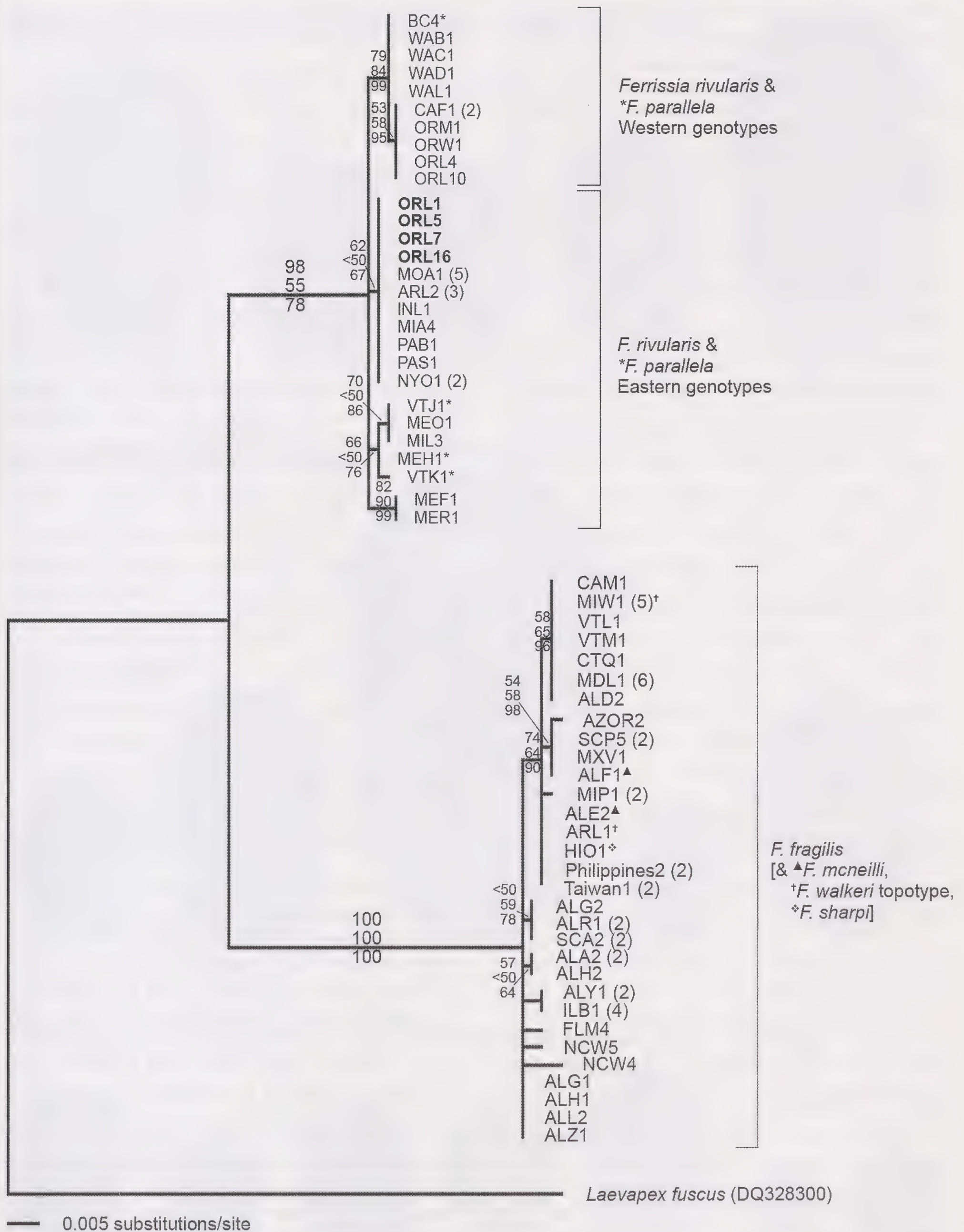


FIG. 5. Bayesian phylogram of the nuclear second internal transcribed spacer (ITS-2) dataset with *Laevapex fuscus* designated as the outgroup. Table 2 has sampling location codes information. If > 1 individual/location bore a genotype, this number is indicated in parentheses. In the *F. fragilis* clade, SCA2 (2) represents the two genotyped limpets sourced from Dillon & Herman's (2009) North Saluda River study population. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented. Four Oregonian specimens that share a *Ferrissia rivularis* Eastern genotype are in bold.

neilli and *F. walkeri*. As in the previous study, the two *Ferrissia* lineages were paraphyletic for this marker, the *F. fragilis* clade being weakly sister with the tip *Ancylus/Rhodacmea* tip clade for maximum parsimony, maximum likelihood and Bayesian analyses. The only other significant addition to the Walther et al. (2006a) 28S dataset was the inclusion of the *Hebetancylus excentricus* genotypes (Fig. 3). This placed within the Laevapecini clade, but sister to South American *Uncancylus* and *Gundlachia* taxa rather than the co-occurring North American *Laevapex fuscus*.

The large nuclear ribosomal (28S) gene is relatively conserved, and much finer-scale phylogenetic resolution can be provided by protein-coding mitochondrial genes (Lee & Ó Foighil, 2005) and, to a lesser degree, by faster-evolving internal transcribed spacer (ITS) regions of the nuclear ribosomal gene complex (Hillis & Dixon, 1991; Lee & Ó Foighil, 2005). We genotyped our limpet samples for mt COI and ITS-2 markers and found that their phylogenetic analyses (Figs. 4, 5) did not change the primary conclusions evident in our 28S ribosomal trees (Fig. 3). Two highly distinctive clades of North American *Ferrissia* were also recovered for each marker; one composed of limpets identified as *F. rivularis* and *F. parallela*, the other containing *F. fragilis* and near-topotypic populations of putative *F. mcneilli* and *F. walkeri*.

The higher resolution provided by mt COI did yield qualitatively new phylogenetic information in the form of conspicuous geographic structuring within the *F. rivularis/parallela* clade (Fig. 4). Both nominal taxa in Pacific drainages formed an exclusive mt COI Western Clade, whereas specimens in eastern drainages formed a corresponding Eastern Clade (Figs. 4, 5). Note that the mt genetic structuring was geographic rather than taxonomic: within each regional clade, individual haplotypes were shared by limpets identified, according to Basch's (1963a) criteria, as *F. rivularis* and *F. parallela* (Figs. 4, 5). The only exception to this regional structuring was one western sampling site (ORL; Table 2) where some *F. rivularis* specimens carrying Eastern Clade haplotypes were recovered in sympatry with Western Clade conspecifics. Although sympatric ORL limpets bearing the respective mt lineages appeared morphologically indistinguishable (Fig. 6), nuclear ITS-2 genotypes corroborate the mt results. *Ferrissia rivularis* and *F. parallela* specimens bearing Western Clade mt genotypes produced an exclusive ITS-2 tip clade (Fig. 5), and there was

agreement among mt and nuclear markers for six ORL *F. rivularis* (2 Western Clade, 4 Eastern Clade) genotyped for both COI and ITS-2.

The mt COI dataset incorporated sequences from a wide variety of ancylinid taxa and one of these, a *Ferrissia* (*Pettancylus*) sp. haplotype sampled from Queensland, Australia (EF012180; Albrecht et al., 2007), was robustly sister to the predominantly *Ferrissia fragilis* clade (Fig. 4). However, basal Ancylini mt COI nodes were poorly supported irrespective of the phylogenetic optimality criterion employed. In maximum parsimony trees, the predominantly *Ferrissia fragilis* clade + *Ferrissia* (*Pettancylus*) sp., was weakly (BS = 55) sister to *F. rivularis* (Fig. 4), although it was weakly (PP = 57) sister to an *Ancylus/Rhodacmea* tip clade in Bayesian and maximum likelihood trees.

Limpets matching *F. fragilis*'s general conchological description (Tryon, 1863; Basch, 1963a) occurring throughout North America in lotic (creeks and rivers) as well as lentic (ponds and lakes) habitats, formed a robustly supported shallow clade (Figs. 3–5). It also included genotyped specimens from near-topotypic populations of putative *F. walkeri* (Benton Co., Arkansas; Berrien Co., Michigan) and putative *F. mcneilli* (Eslava River and Eslava Lake, Alabama), both of which shared individual mt COI haplotypes with *F. fragilis* specimens (Fig. 4). Comparison of our genotyped Eslava specimens with those collected there by McNeill and identified by Walker as *F. mcneilli* showed that they possessed overlapping shell phenotypes (Fig. 7).

The predominantly *F. fragilis* clade lacked obvious geographic structuring, and Alabama samples were present throughout the mt COI and nuclear ITS-2 topologies (Figs. 4, 5). A notable feature, however, was the presence of multiple founder populations in Europe and Asia (Figs. 4, 5), discussed in detail by Walther et al. (2006b). Our novel data detected two additional founder populations on oceanic island archipelagoes: Azorean *Ferrissia* and our single specimen of *F. sharpi*. The latter taxon, a Hawaiian putative endemic, shared a mt COI haplotype with multiple Californian *F. fragilis* specimens, though it was distinct from the shared haplotype linking another subset of Californian *F. fragilis* with founder populations in Taiwan and the Philippines (Fig. 4).

Clade-Specific Shell Phenotypes

Although all three genes employed readily differentiated the two main lineages of *Fer-*

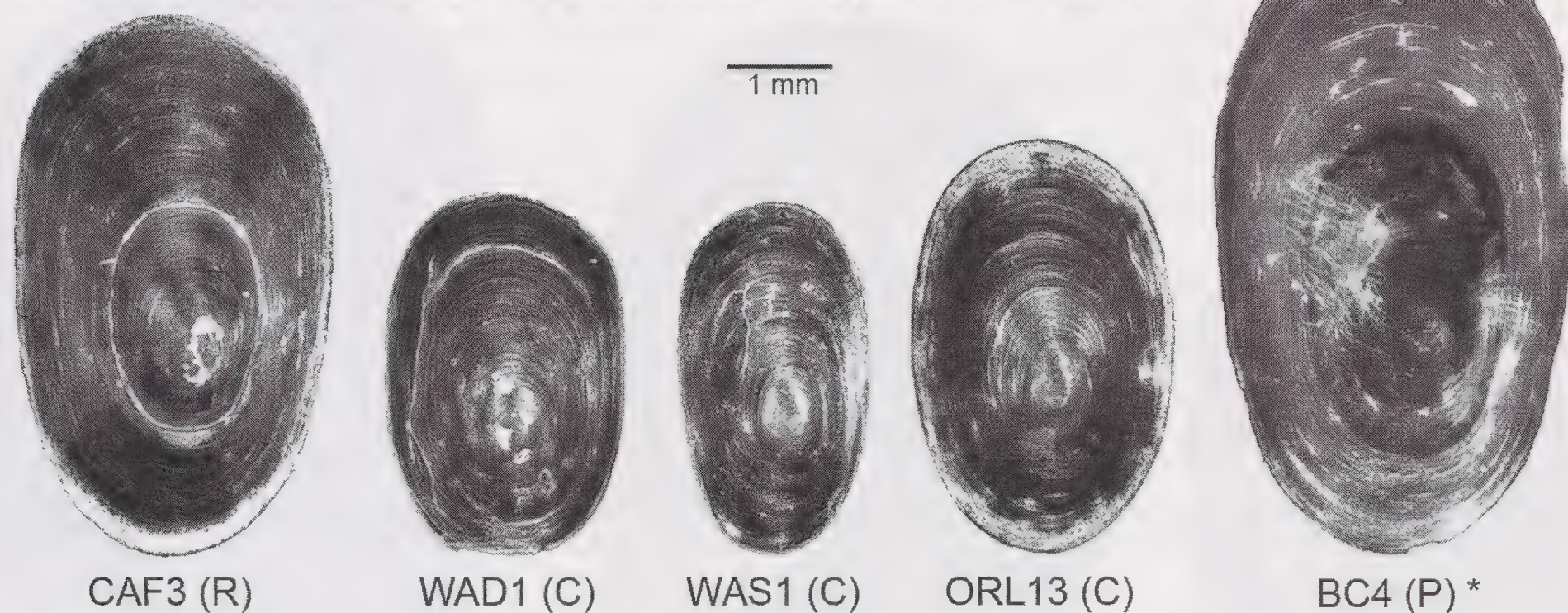
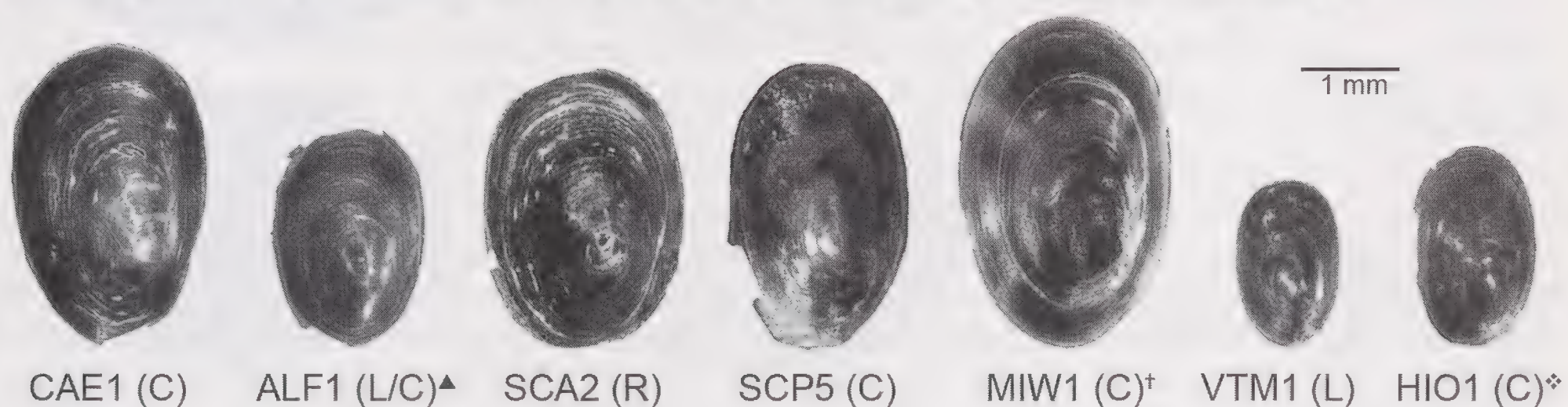
Ferrissia rivularis* & **F. parallela* - Western clade**F. rivularis* & **F. parallela* - Eastern clade*****F. fragilis*, [▲]*F. mcneilli*, [†]*F. walkeri* topotype, & [✧]*F. sharpi***

FIG. 6. Photographs of representative shell vouchers arranged according to their topological placement in mt COI and ITS-2 gene trees (Figs. 4, 5). Table 2 has corresponding location codes. Letters in parentheses accompanying the codes indicate habitat sampled: R = river, C = creek or stream, L = lake, P = pond, L/C = creek mouth emptying into a lake.

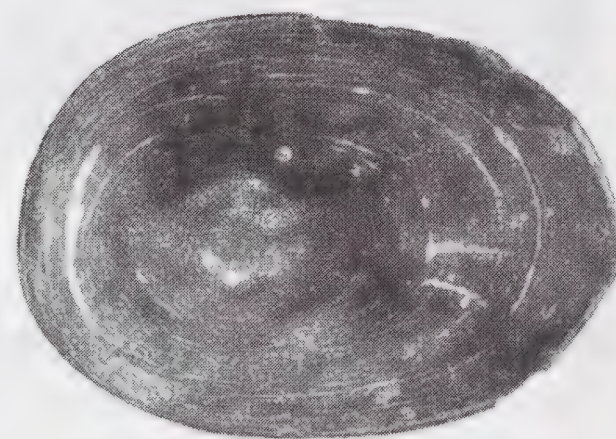
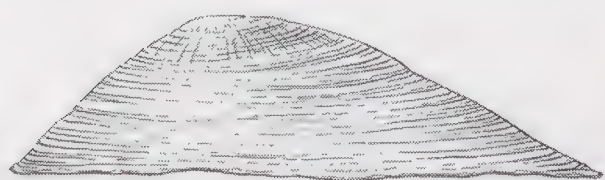


FIG. 7. Comparative photographs of a representative *Ferrissia mcneilli* specimen sampled by L. H. McNeill at Eslava Creek, Alabama, and identified by Walker (UMMZ 161631), juxtaposed with two genotyped Eslava Creek, Alabama, limpets sampled by the authors and identified by them as *F. fragilis*.

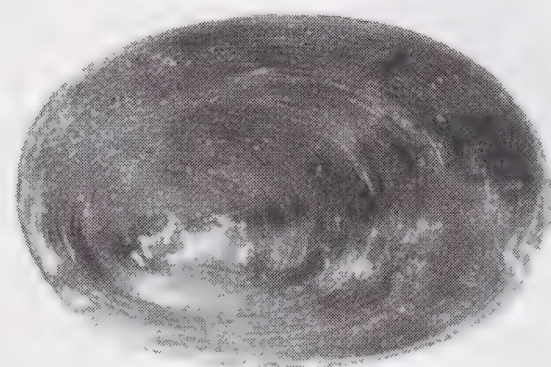
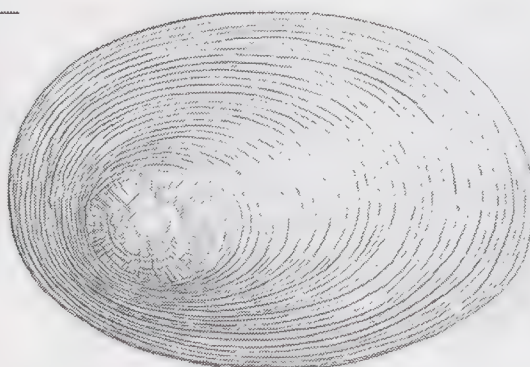
rissia in North America (Figs. 3–5), we were interested in identifying morphological attributes that could more conveniently serve this purpose. Differentiating adult members of the *F. rivularis*/*F. parallella* clade was easy because their maximum body size (≤ 9 mm in length; Fig.

6) was much larger than the predominantly *F. fragilis* clade members we genotyped (≤ 3.5 mm in length; Fig. 6), consistent with Tryon's (1863) description of *F. fragilis* as being "much the smallest of our species". This still leaves the difficulty of identifying the cladal affinities

F. rivularis



F. fragilis



1 mm

FIG. 8. Comparative drawings (lateral and dorsal views) and photographs (dorsal views) of representative similar-sized *Ferrissia rivularis* and *F. fragilis* specimens that were sampled sympatrically from a Crystal Lake tributary in Benton Co., Arkansas (ARL) and then genotyped (Figs. 3, 5). Note that the *F. fragilis* specimen was distinguished by an eccentric apex that was depressed, positioned posterior to the shell summit, and posteriad.

TABLE 3. Apical descriptions of the nominal taxa present in our predominantly *Ferrissia fragilis* clade (Figs. 3–5).

Taxa	Salient Apex Characteristics	Author
<i>F. fragilis</i>	“elevated, acute, curved backwards, with about 2/3rds of the shell anterior to it”	Tryon, 1863
<i>F. walkeri</i>	“excentric...decidedly more so than in <i>A. rivularis</i> ...summit of the shell is in front of the somewhat depressed apex”	Pilsbry & Ferriss, 1907
<i>F. michiganensis</i> (syn. <i>F. walkeri</i> by Basch, 1963a)	“the greatest height being about in the center of the shell, from which point it slopes slightly towards the apex....apex slightly depressed, excentric, turned toward the right side”	Winslow, 1923
<i>F. mcneilli</i>	“prominent, sub-acute, somewhat obtuse, eccentric...” (lateral-view figure shows that summit of shell is in front of depressed apex)	Walker, 1925
<i>F. sharpi</i>	“well behind the middle and well to the right of the median line...inclined to the right and posteriad” (lateral-view figure shows that summit of shell is in front of depressed apex)	Hubendick, 1967

of smaller-bodied (≤ 4 mm in length) North American *Ferrissia* specimens.

Detailed descriptions of the nominal taxa present in our predominantly *Ferrissia fragilis* clade (Figs. 3–5) agree on a number of potentially useful distinguishing features of the shell apex (Table 3). These include a more pronounced apex eccentricity (relative to *F. rivularis*) in addition to a depressed/posteriad orientation associated with its positioning posterior to the shell summit. These features, especially the latter, are apparent in the type material of nominal *F. fragilis*, *F. walkeri*, and *F. mcneilli* (Fig. 2), as well as in our voucher material of genotyped *F. fragilis*, *F. sharpi*, and near-topotypic putative *F. walkeri* and *F. mcneilli* (Fig. 6). Although this appeared promising, the most meaningful test necessarily entailed sympatric populations: could we predict *Ferrissia* cladal membership based on apical differences of co-occurring similarly-sized limpets? Two of our sampling sites (ARL and MAS) contained limpets from both clades (Fig. 1; Table 2) and two distinctions were evident among their respective shell phenotypes. First, *F. fragilis* clade member shells were (as the specific name implies) more fragile than that of sympatric *F. rivularis* clade members, and many of the former were inadvertently damaged by routine handling during sampling and/or tissue extraction. Second, although there was some minor individual variation, adult *F. fragilis* clade members had apices (protoconchs) that were typically positioned just posterior to the shell summit (at the shell summit for *F. rivularis* clade

members) and that were consistently more eccentric, depressed and posteriad in orientation than those of similarly-sized sympatric *F. rivularis* clade members (Fig. 8).

DISCUSSION

Our primary phylogenetic result was consistent for all three genetic markers: two highly distinctive *Ferrissia* clades, each encompassing multiple nominal species, were widely distributed across North American watersheds. In a major departure from Basch’s (1963a) taxonomic scheme, both clades lacked discrete habitat demarcations and occurred in both lentic and lotic habitats.

Nominal *Ferrissia rivularis* and *F. parallela* specimens shared individual genotypes for all three markers and exhibited geographic, rather than taxonomic, genetic structuring. Our data therefore strongly indicated that the two nominal taxa represent lotic (*F. rivularis*) and lentic (*F. parallela*) ecophenotypes of the same limpet species. *Ferrissia rivularis* (Say, 1817) has priority over *F. parallela* (Haldemann, 1841), and we recommend that the latter taxon be reclassified as a *F. rivularis* junior synonym. This result was not entirely surprising, because Basch (1983a) regarded *F. parallela* as “a direct descendant of *F. rivularis*”. However, we cannot be confident that lentic environments represent derived habitats for *F. rivularis*, because, at present, we lack convincing sister lineages for this North American species that would allow

us to infer a plesiomorphic habitat type.

The recovery of east-west genetic structuring in our sampled *F. rivularis* populations for mt COI and, to a lesser extent, for nuclear ITS-2, was consistent with the presence of a long-term barrier to gene flow, presumably along the continental divide. Nevertheless, our discovery of limpets bearing Eastern genotypes in one of the Western populations indicated that this barrier has been secondarily breached in an east-to-west direction, possibly *via* human agency. Preliminary genetic characterization of the genetically mixed east/west population (ORL) did not recover evidence of introgression, for example, cyto/nuclear disjunction. However, this does not necessarily imply that the two morphologically indistinguishable lineages are reproductively incompatible because our sample sizes were small and some ancylinids reproduce predominantly by self-fertilization (Städler et al., 1993, 1995; Dillon & Herman, 2009). The geographically widespread European ancylinid *Ancylus fluviatilis* encompasses a cryptic species complex containing multiple reproductively isolated lineages (Pfenniger et al., 2003; Albrecht et al., 2006). Future in-depth research of continental *F. rivularis* populations may well support a similar conclusion, but at the present state of our knowledge, we consider it most useful to classify the observed east-west genetic structuring as within-species phylogeographic variation.

Nominal *Ferrissia fragilis*, *F. sharpi* and putative *F. mcneilli* and *F. walkeri* specimens shared individual genotypes (Figs. 3–5) and exhibited overlapping shell phenotypes (Figs. 6, 7; Table 3). These results led us to conclude that they are all conspecific and to reclassify *F. sharpi* (Sykes, 1900) and *F. walkeri* (Pilsbry & Ferriss, 1907) and *F. mcneilli* Walker, 1925, as junior synonyms of *F. fragilis* (Tryon, 1863).

Ferrissia fragilis has a most interesting, multi-layered biogeography. Our mt COI gene trees recovered a robust sister relationship with an Australian *Ferrissia* (*Pettancylus*) sp. (Fig. 4), thereby corroborating the preliminary Old World phylogenetic linkage of *F. fragilis* with an African sample of *Ferrissia* (*Pettancylus*) cf. *clessiniana* obtained for a different mt marker (Walther et al., 2006b, c). Within North America, *F. fragilis*, unlike *F. rivularis*, showed no strong evidence of regional cladogenic structuring, for example, the most common mt COI haplotype was present in Alabama, Michigan, and California samples (Fig. 4). However, the observed genetic diversity was unevenly distributed: southeastern (Alabama) samples were the most diverse and encompassed genotypes that placed throughout the topologies of ITS-2 and mt COI gene tree/network topologies (Figs. 4, 5). Another notable feature was the presence of a discrete subsample of North American mt COI haplotypes, or near-mutational derivatives, in European, Asian and oceanic island founder

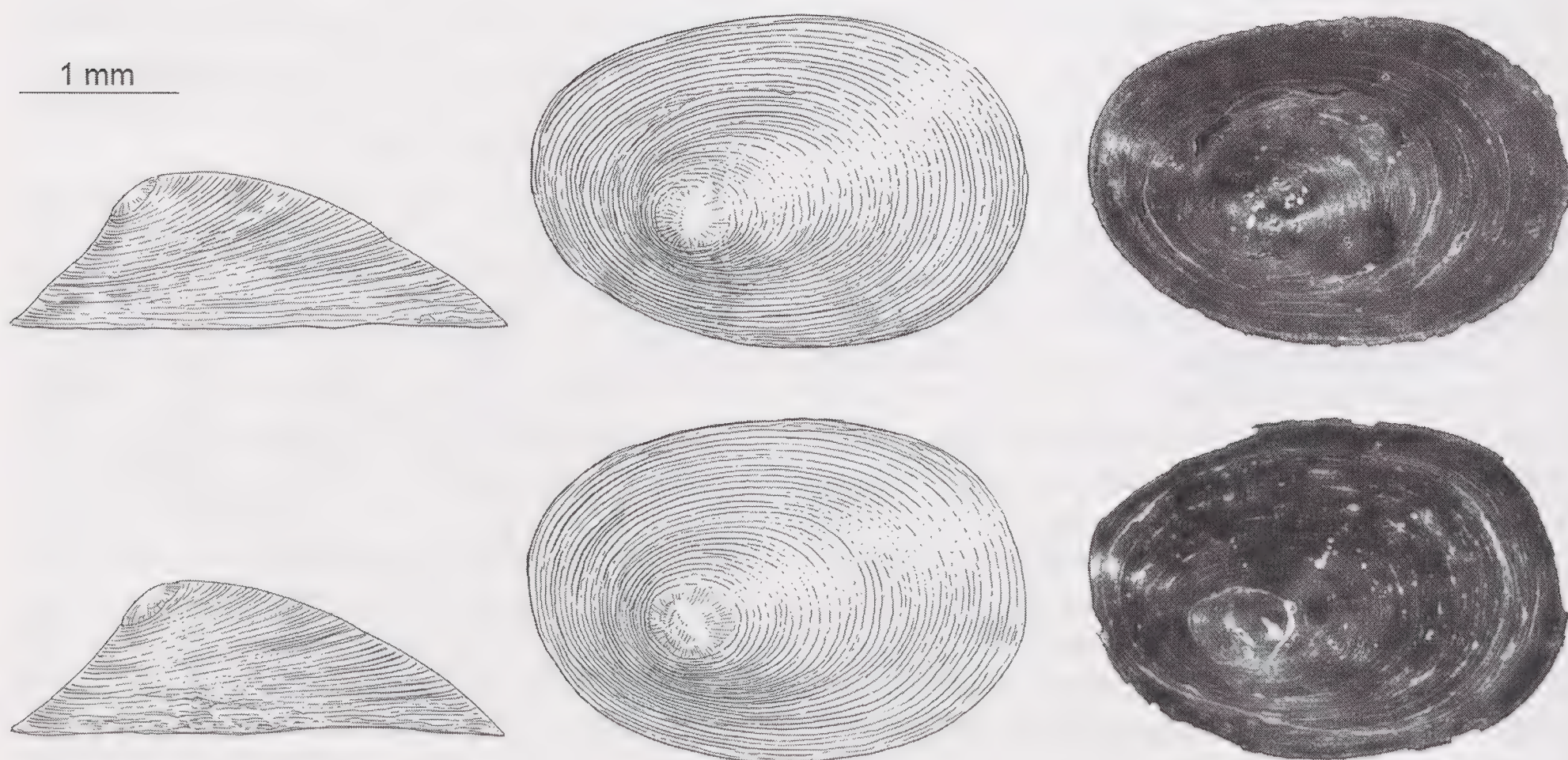


FIG. 9. Drawings (lateral and dorsal views) and photographs (dorsal views) of the shells of two genotyped (SCA2; Figs. 3, 5) North Saluda River *F. fragilis* specimens. Note that they display eccentric, depressed, posteriad apices that are positioned posterior to the shell summit.

populations (Fig. 4) that apparently stem from historically recent cryptic anthropogenic invasions (Walther et al., 2006b, c). In some cases, our expanded sampling of North American populations allowed us to identify putative regional source populations, for example, Hawaiian and Asian (Taiwan, Philippines) founder populations exclusively shared (different) haplotypes with Californian conspecifics (Fig. 4).

Putting aside recent globally invasive founder populations, the deeper phylogenetic Old World ties of *Ferrissia fragilis* to Australian and African congeners (Fig. 4; Walther et al., 2006b, c) ostensibly contradict Hubendick's (1964) distinction of New World *F. (Ferrissia)* from Old World *F. (Pettancylus)* subgenera. However, Hubendick's (1964) anatomical analysis of New World *Ferrissia* taxa was restricted to *F. rivularis* and *F. parallela*, and he apparently did not examine North American populations of *F. fragilis*. Nevertheless, he did study what we now know to be cryptic invasive *F. fragilis* founder populations in Hawaii (Hubendick, 1967; identified as *F. sharpi*) and in Europe (Hubendick, 1964; identified as *Watsonula wautieri*), both of which he assigned to *Pettancylus*. This agreement among molecular phylogenetic (Fig. 4; Walther et al., 2006b, c) and morphological diagnoses (Hubendick, 1964, 1967) concerning *F. fragilis*' systematic affinities allow us to group it with the Old World subgenus *Pettancylus*. However, this necessitates a subgeneric name change because Hannibal (1912) used *F. fragilis* as the type species for his new subgenus *Kincaidilla*, and this has precedence over *Pettancylus* Iredale 1943. The *Ferrissia* subgenus *Kincaidilla* notably unites, for the first time, New World and Old World septum-forming *Ferrissia* taxa.

Our systematic conclusions are obviously incongruent with Dillon & Herman's (2009) recent synonymization of *F. rivularis* and *F. fragilis* based on allozymic and morphometric analyses of two South Carolina populations. They had kindly forwarded exemplar specimens to us for genotyping, and it is important to stress that their allozyme analyses concurred with our DNA phylogenies: both populations were found to be conspecific. The disagreement centers on the taxonomic identity of one of their two study populations: North Saluda River.

Dillon & Herman (2009) identified their North Saluda River study population as *F. rivularis* partially on ecophenotypic conchological features but also on the basis of habitat (a fast flowing stream segment). However, *F. fragilis* population-level conchological phenotypes

span a wide vertical spectrum from depressed to elevated (Basch 1963a: fig. 18), and we now know that this species occurs in many lotic habitats across the continent (Table 2; Fig. 6). Dillon & Herman (2009) justified their synonymization of *F. rivularis* and *F. fragilis* on the presumed absence of any reliable morphological distinction separating these two taxa. Nevertheless, a suite of morphological distinctions including maximum body size (Basch, 1963a; Fig. 6), details of apical positioning and orientation (Table 3; Fig. 8), egg capsule characteristics (Basch, 1959), and the inducible ability to form a septum (Basch, 1963a; Richardot, 1974, 1977a, b), give grounds for cautious optimism that it should be possible to distinguish sympatric populations without resorting to genotyping.

The available morphological data (body size, egg capsule characteristics, conchology) for North Saluda River specimens were consistent with their phylogenetic identity (Figs. 3, 5). For instance, their maximum length of 3.4 mm, (Dillon & Herman, 2009), fell comprehensively short of that expected for a *Ferrissia rivularis* adult year class (Basch, 1963a: fig. 13). Their egg capsules exclusively contained single eggs (Dillon & Herman, 2009), a characteristic of *F. fragilis* (Basch, 1959; Mirolli, 1960; Walther et al., 2006b) but not of *F. rivularis* and *F. parallela* (Basch, 1959; Burky, 1971; Clarke, 1973). Finally, they clearly displayed the apical characteristics of *F. fragilis* (Fig. 9). In conclusion, the available phylogenetic, morphological and reproductive characters concur that Dillon & Herman's (2009) North Saluda River study population was *F. fragilis*, not *F. rivularis*, and we are therefore unable to support the proposed synonymization of these two taxa.

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